

[54] **SALTS OF NOVEL
p-DIHYDROXYBENZENE DISULFONIC
ACIDS**

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- [63] Continuation-in-part of Ser. No. 359,771, May 14,
1973, abandoned.

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- [58] **Field of Search** 260/268 B, 268 R, 501.21,
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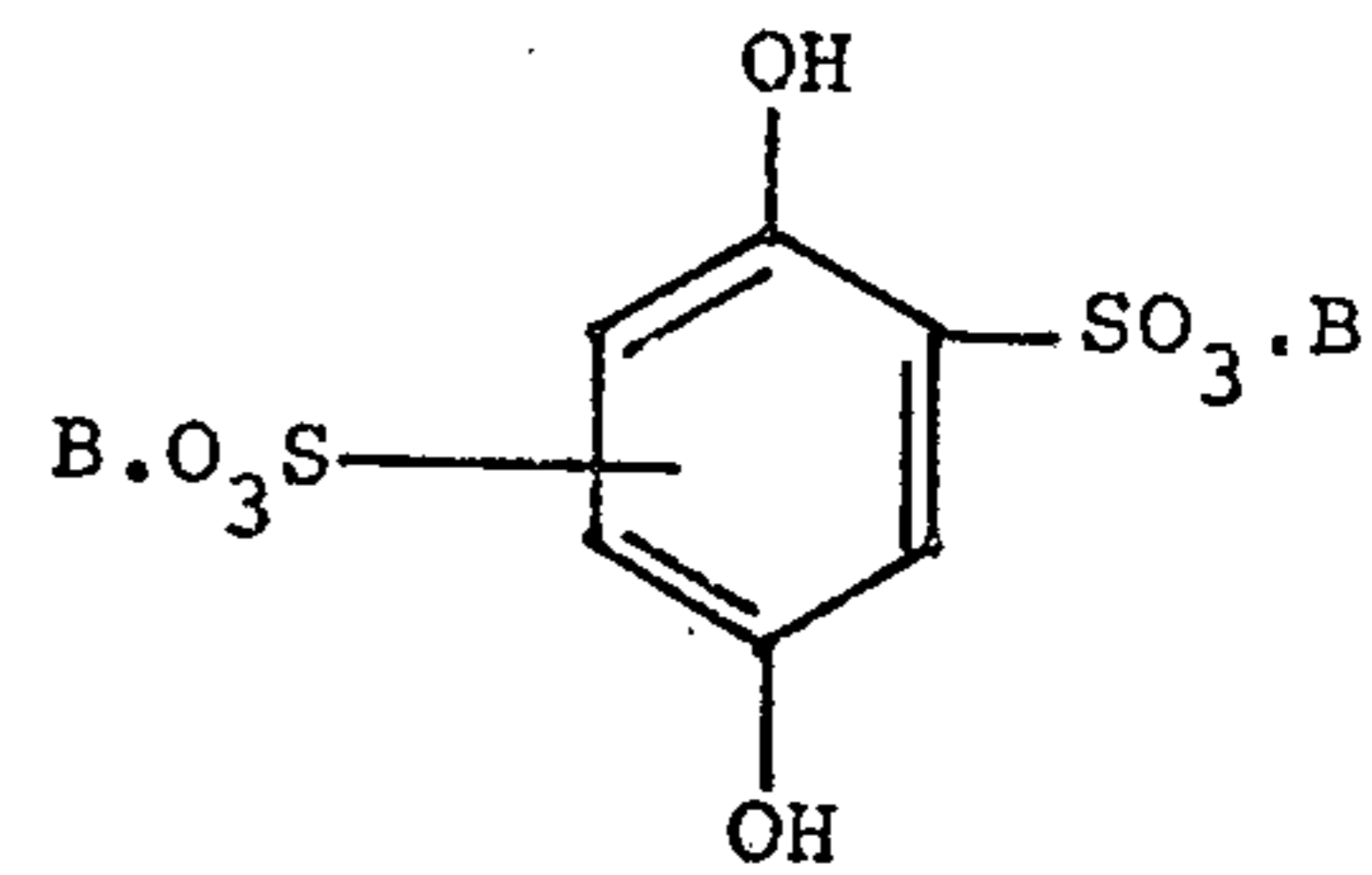
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[57] **ABSTRACT**

Salts of p-dihydroxy benzene disulfonic acids having
the general formula:



wherein B is an alkali metal or an equivalent of an al-
kaline-earth metal, or BH is the cation of ammonia or
a nitrogen containing basic organic compound, are
useful for reducing the average bleeding time and for
combating capillary fragility.

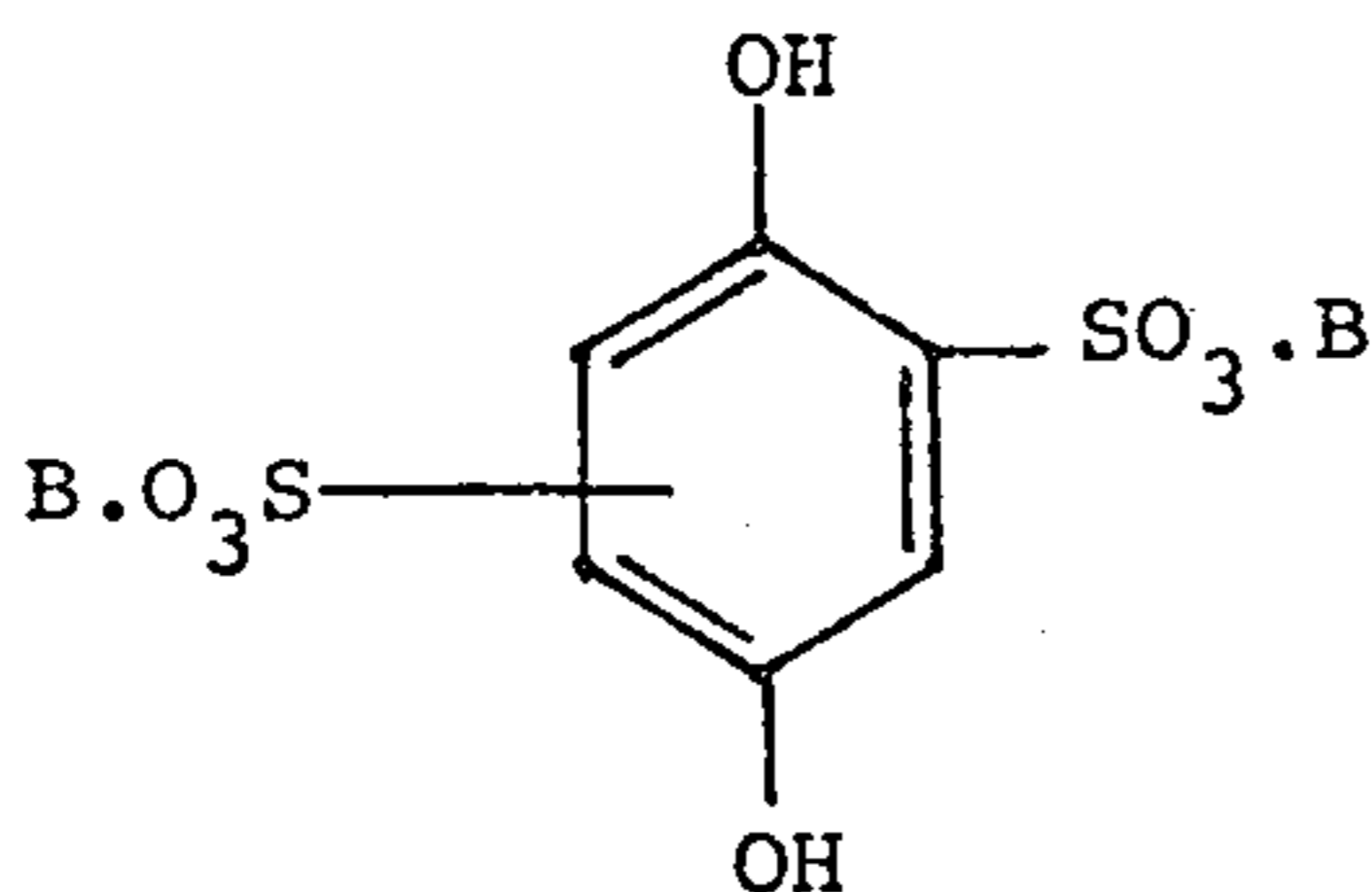
8 Claims, No Drawings

SALTS OF NOVEL P-DIHYDROXYBENZENE DISULFONIC ACIDS

This application is a continuation-in-part of my co-
pending application Ser. No. 359,771 filed May 14,
1973, now abandoned.

This invention concerns novel salts of p-dihydroxy
benzene disulfonic acid, and a process for preparing
said salts.

The compounds of the invention have the general
formula:



wherein B indicates an alkali metal, particularly lith-
ium, or an equivalent of an alkaline-earth metal, partic-
ularly calcium, or BH is the cation of a nitrogen-con-
taining basic compound such as ammonia, alkyl, alka-
nol, aryl, alkylaryl amines, cyclo-amines, and generally
all the nitrogen-containing basic compounds capable of
forming salts with p-dihydroxy benzene disulfonic acid.

The compounds of the invention are useful as hemo-
static agents, and for protecting capillary fragility.

The activity for the hemostatic action is determined
in vivo, by ROSKAM's method, as modified by LA-
PORTE, on the rabbit's ear, and the protective action
on the capillaries by the method of BEACH and STEI-
NETZ. The therapeutical index (ratio between LD₅₀
and TE₅₀) is very favorable, in view of the high activity
and low toxicity.

The process of preparation according to this inven-
tion is characterized in that a p-dihydroxy benzene
disulfonic acid is neutralized with a base of the cation
of formula B, or in that an exchange reaction is effected
between a metal salt of p-dihydroxy benzene disulfonic
acid, and a salt of the B cation with another acid. Pref-
erably, the starting p-dihydroxy benzene disulfonic acid
is free from impurities such as small amounts of metal
elements. In order to obtain this condition, one has
studied a novel method for preparing it, which is part of
this invention, from salts of p-dihydroxy benzene
monosulfonic acid. To do so, it is necessary to first
dehydrate said salt by heating it with liquids forming an
azeotrope with water, and by distilling the amount of
liquid required to obtain the anhydrous mixture of the
salt and solvents. Subsequently, sulfonation is carried
out by treating the mixture with the sulfonating acid,
so that the sulfate of the salt formed, e.g. potassium
sulfate or calcium sulfate, can be separated from the
mixture when the reaction is completed. From the
aqueous, alcoholic, or a solution, of the p-dihydroxy
benzene disulfonic acid in any other solvent, thus
formed and separated from the insoluble salt, the salts
of said acid can be obtained either by neutralization or
by a salt exchange reaction.

With the appropriate salt, whatever it may be, the
following Examples illustrate the preparation of the
compounds of this invention.

EXAMPLE 1

bis(methylamine) p-dihydroxy benzene disulfonate

a. Preparation of p-dihydroxy benzene disulfonic
acid:

190 g of p-dihydroxy benzene monosulfonic acid,
100 ml of dioxane and 100 ml of perchloroethylene are
mixed in a flask provided with a reflux condenser. The
flask is heated in an oil bath to the reflux temperature
(140°C in the oil bath). It is refluxed for half an hour,
and then it is left to cool a little to set the condenser for
distillation. Two layers are formed in the flask: a co-
lourless lower layer, and a coloured upper layer in
which the acid is dissolved. The flask is then heated
again, and 100 ml of the mixture of solvents are dis-
tilled. In the mixture collected, two layers are formed;
the upper one, which is moderate is aqueous. To the
flask provided with a reflux condenser, 98 g of sulfuric
acid containing 20 % of SO₃ are added, and it is heated
again, at a lower temperature, so that the reflux is
barely noticeable (temperature of the bath: 130°C), for
four hours. The liquids which accompany the acid are
removed by reduced pressure distillation, the residue is
dissolved in a little water, 2 g of active charcoal are
added, it is filtered. The aqueous solution can be used
as such for obtaining the respective salts.

b. Preparation of the methylamine salt:

By adding a methylamine solution to the aqueous
solution of the acid prepared as indicated, the methyl-
amine salt is obtained in solution. This solution is
treated with active charcoal, filtered, and vacuum-con-
centrated, so as to obtain a crystalline residue of bis
(methylamine) p-dihydroxy benzene disulfonate
which, after recrystallizing in water, provides 167 g of
pure product, having a melting point of 288°C.

EXAMPLE 2

bis (ethanolamine) p-dihydroxy benzene disulfonate

Neutralization of the acid resulting from the previous
operation with 122 g of ethanolamine, followed by
filtration and recrystallization, provides 211 g of bis
(ethanolamine) p-dihydroxy benzene disulfonate, hav-
ing a melting point of 182°C.

EXAMPLE 3

bis (phenylamine) p-dihydroxy benzene disulfonate

a. Preparation of potassium p-dihydroxy benzene
disulfonate:

Neutralization of p-dihydroxy benzene disulfonic
acid, obtained as indicated in Example 1, with an aque-
ous solution of potassium hydroxide, gives the potas-
sium salt of said acid. This salt crystallizes and can
easily be purified to give an extremely pure product:
C₆H₄O₈K₂S₂ M.W. = 346, without water of crystalliza-
tion, which decomposes above 270°C.

b. Preparation of the phenylamine salt:

346 g of the potassium salt, prepared as described
above, are dissolved in warm water. Since the solubility
of this salt is not high, a liter of distilled water, kept at
a temperature of 80°-90°C must be used. On the other
hand, in order to prepare the corresponding bitartrate,
a solution of 300 g of anhydrous tartaric acid is neutral-
ized with the required amount of aniline, i.e. 186 g of
freshly distilled product. The latter solution is poured
onto the potassium salt solution, heating and stirring
being maintained for 15 minutes. It is left aside to cool,

and is left overnight in a refrigerator, the following day the precipitate of potassium bitartrate is filtered off, and the solution is concentrated until it begins to crystallize. After vacuum filtering the solid portion, the product is recrystallized until pure, melting point above 300°C (dec).

EXAMPLE 4

bis (piperazine) p-dihydroxy benzene disulfonate

a. Preparation of the potassium salt solution:

As in the preceding Example, 346 g of the potassium salt of p-dihydroxy benzene disulfonic acid are dissolved in 1 liter of distilled water, and the solution is kept warm to prevent crystallization.

b. Preparation of piperazine perchlorate:

To an aqueous solution of perchloric acid, containing 200 g of said acid in 500 ml of water 40 % solution, the amount of aqueous solution of piperazine hexahydrate required for neutralization is added. The required amount of piperazine hexahydrate is 388.46 g ($C_4H_{10}N_2 \cdot 6H_2O$) dissolved in 500 ml of water. Preferably the pH of the neutralizing solution is controlled to a pH close to 6. The piperazine perchlorate solution is mixed with the warm solution of p-dihydroxy benzene disulfonic acid potassium salt, and is stirred until a copious precipitate of potassium perchlorate is formed, which then increases when the solution is left to cool for several hours. After filtering off the potassium perchlorate, the solution of a piperazine p-dihydroxy benzene disulfonate is concentrated in a rotary evaporator, under vacuum, at 60°C, until crystals begin to form, and it is then left in a refrigerator. The product is filtered and recrystallized in water to give 388 g of bis (piperazine) p-dihydroxy benzene disulfonate, having a melting point of 270°C.

EXAMPLE 5

lithium p-dihydroxy benzene disulfonate

a. Preparation of p-dihydroxy benzene disulfonic acid:

190 g of p-dihydroxy benzene monosulfonic acid, 100 ml of dioxane, and 100 ml of perchloroethylene are mixed in a flask provided with a reflux condenser. The flask is heated in an oil bath to the reflux temperature (in the oil bath at 140°C). It is refluxed for half an hour, and then is left to cool a little to set the coolant for distillation. In the flask, two layers are formed: a colourless lower layer, and a coloured upper layer in which the acid is dissolved. The flask is heated again and 100 ml of the solvent mixture are distilled. In the mixture collected, two layers form, the upper, moderate, is aqueous. To the flask provided with a reflux condenser, 98 g of sulfuric acid containing 20 % of SO_3 are added, and it is heated again, to a lower temperature, so that the reflux is barely noticeable (bath temperature: 130°C), for four hours. The liquids accompanying the acid are removed by distilling at reduced pressure, the residue is dissolved in a little water, 2 g of active charcoal are added, and it is filtered. The solution is concentrated, cooled, and is precipitated by adding five volumes of acetone. The precipitate is filtered, and 189 g of p-dihydroxy benzene disulfonic acid, with a melting point of 166° - 169°C are obtained.

b. Preparation of the lithium salt:

270 g of acid obtained according to (a) are dissolved in 2 liters of boiled and oxygen-free distilled water, and the solution is carefully neutralized with 65 g of pure

lithium carbonate, the pH being maintained between 6 and 6.5. After filtering the solution is concentrated with the precautions described in paragraph (a), and it is poured into a crystallising dish as soon as the first crystals appear. After cooling, and separating the mother liquors, 300 g of product are obtained which are recrystallized in water. 270 g of lithium p-dihydroxy benzene disulfonate are obtained, having a melting point above 260°C (dec). The infrared spectrum recorded in a KBr pellet gives maxima at the following frequencies: 3480, 3360, 3210, 1690, 1425, 1260, 1205, 1165, 1110, 1035, 885, 825, and 670 cm^{-1} .

EXAMPLE 6

calcium p-dihydroxy benzene disulfonate

a. Preparation of p-dihydroxy benzene disulfonic acid:

41.6 g diethylamine p-dihydroxy benzene disulfonate (obtained by a known method, but particularly according to Example 1 of Swiss patent application Ser. No. 7326/72), are dissolved in 500 ml of air-free distilled water. The solution thus obtained is passed through a column of cation exchange resin (Amberlite IRC-50) containing 60 g of resin prepared in acid form and washed. The liquids are collected in a flask protected from the air (nitrogen atmosphere), and the resin is washed until the eluant no longer gives the reaction of phenols with the ferric chloride solution, then the washings are combined with the main solution. The combined liquids are concentrated in a rotary evaporator, under vacuum, until the solution becomes cloudy, i.e. until the total volume is about 80 ml. When the solution is cold, and before the product crystallizes, 200 ml of pure acetone are added; a crystalline mass is formed which is collected in a Buchner funnel under slight vacuum. After recrystallization in water, 27 g of extremely pure acid are obtained, having a melting point of 166° - 169°C.

b. Preparation of the calcium salt:

270 g of the acid obtained according to part (a) are dissolved in 2 liters of boiled, oxygen-free distilled water, and the solution is carefully neutralized with 100 g of pure calcium carbonate, the pH being maintained between 6 and 6.5. The solution is filtered, vacuum concentrated, and left to cool. A crystalline residue of calcium p-dihydroxy benzene disulfonate is obtained which is filtered and washed successively with distilled water, alcohol, and finally with diethyl ether, giving 157 g of pure product having a melting point above 300°C (dec). the infrared spectrum recorded in a KBr pellet gives maxima at the following frequencies: 3500, 1625, 1420, 1340, 1220, 1175, 1110, 1025, 885, and 810 cm^{-1} .

EXAMPLE 7

bis (ammonium)-p-dihydroxy benzene disulfonate

The aqueous solution of p-dihydroxy benzene disulfonic acid, obtained according to example 1, is made colorless by filtering after addition of 2 g of charcoal, and then concentrated by evaporation under reduced pressure, until a granular paste is obtained. 100 ml of ether added, and the thus formed suspension is filtered through a Buchner funnel by suction. The paste obtained is dissolved in 200 ml of distilled water, the solution is filtered and neutralized with concentrated ammonia. After each addition of a portion of ammonia, the pH of the solution is checked, and ammonia addi-

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tion is stopped at a pH of 6.5. The solution then becomes unclear, and the ammonium salt begins to precipitate.

Without removing the solid particles, the solution is heated to boiling, 1 g of charcoal is added, and the hot solution is filtered through a paper filter. The filtrate is cooled down during several hours, and the precipitated ammonium salt is removed by filtering. After several recrystallizations from water, one obtains 92 g of bis (ammonium) p-dihydroxy benzene sulfonate, in the form of a white crystalline powder.

The mother liquids are concentrated, and after recrystallization as above described, one obtains 9 g of the same ammonium salt, having a melting point above 320°C. The IR-spectrum measured in KBr gives at 1530, 1410, 1210, 1115, 1020, 890, 820 and 660 cm^{-1} .

OTHER PRODUCTS OBTAINED BY THE PROCESS DESCRIBED

bis (dimethylamine) p-dihydroxy benzene disulfonate	M.P.	199°C
bis (diethylamine) p-dihydroxy benzene disulfonate	M.P.	198-220°C
bis (trimethylamine) p-dihydroxy benzene disulfonate	M.P.	260°C
bis (pyrrolidine) p-dihydroxy benzene disulfonate	M.P.	272°C

The compounds of the invention are interesting as hemostatic agents, for their protective action on the capillaries, and as antilipedemic agents.

The pharmacodynamical properties of the products which are the object of this patent are typified by those of bis-diethylamine-p-dihydroxy-benzene-disulfonate.

1. Acute toxicity in the mouse and the rat

18 to 25 g albino mice

100 to 150 g Sprague-Dawley rats

The LD_{50} was determined according to the method of Reed and Muench.

TABLE I

Administration method	Species	LD_{50} (mg/kg)	Fiducial limits (for $p = 0,95$)
I.V.	mouse (male)	1,016	(831 - 1,525)
I.V.	mouse (female)	1,016	(831 - 1,525)
Oral	mouse (male)	6,292	(5,330 - 7,423)
Oral	mouse (female)	5,878	(5,286 - 6,532)
I.P.	rat (male)	2,650	(2,307 - 3,041)
I.P.	rat (female)	2,442	(2,127 - 2,804)
Oral	rat (male)	5,837	(4,425 - 7,692)
Oral	rat (female)	4,678	(3,978 - 5,493)

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2. Action on the average bleeding time

bis-diethylamine p-dihydroxy benzene disulfonate produces a decrease of the bleeding time in the rabbit, determined by the method of ROSKAM, modified by LAPORTE (Chemotherapia, 3, 62, 1961). The results obtained one hour after administering the product are given in Table II.

TABLE II

Dose micromoles/kg	Effect Percentage decrease of average bleeding time
0.625	8.5 %
1.25	19.5 %
2.5	32.0 %
5.0	43.0 %
7.05	51.0 %
10.0	50.0 %

3. Activity: time ratio

The effect of bis-diethylamine p-dihydroxy benzene disulfonate at a dose of 5 micromoles/kg is long lasting, as can be seen from the results given in Table III.

TABLE III

Time hours after i.v. administration	Effect % decrease of average bleeding time
1	43.0 %
2	46.8 %
4	42.0 %
8	33.5 %
16	32.0 %
24	21.0 %

4. Protective action on capillaries

The action of bis diethylamine p-dihydroxy benzene disulfonate on the capillary permeability for the mouse was determined by the modified method of BEACH-STEINITZ (J. Pharmacol. Exp. Ther. 131 (1), 400, 1961). The product, administered i.p., causes a decrease of the capillary permeability which appears after 8 micromoles/kg, and higher doses, and the maximum effect is attained at a dose of 200 micromoles/kg. Intermediate doses produce effects proportional to the logarithm of the dose.

5. Hypolipedemic action in the rat

Bis-diethylamine-p-dihydroxy-benzene disulfonate significantly inhibits the increase of the plasma cholesterol, triglyceride, and total lipids levels in Sprague-Dawley rats treated with Triton WR-1339 (Friedmann M. and Byers S.O., J. Exptl. Med., 97, 117, 1953). The results obtained are given in Table IV.

TABLE IV

	TRITON	TRITON + BIS DIETHYLAMINE P-DIHYDROXY BENZENE DISULFONATE
TOTAL CHOLESTEROL mg % (plasma)	238.4 ± 6.5	223.0 ± 15.1
Δ % with respect to Triton		- 6 %
P		0.15 < P < 0.20
FREE CHOLESTEROL mg % (plasma)	60.5 ± 2.5	54.4 ± 3.9
Δ % with respect to Triton		- 10 %
P		0.05 < P < 0.10
TRIGLYCERIDES mg % (plasma)	773.5 ± 59.1	589.5 ± 57.9
Δ % with respect to Triton		- 24 %
P		0.0125 < P < 0.025
TOTAL LIPIDS mg % (plasma)	2466.9 ± 141.2	2001.9 ± 193.7
Δ % with respect to Triton		- 19 %
P		0.025 < P < 0.05

The following table gives the values for DL₅₀ and DE₅₀, as well as proposed doses of some compounds according to the present invention.

	DL ₅₀ (mg/kg)	DE ₅₀ (μmoles/kg)	Proposed dose in g/day		
			Rectal	Oral	Parenteral
bis (piperazine) p-dihydroxybenzene disulfonate (p.o.)	>2000	1,25	1 - 2	1 - 2	0,25 - 1
lithium p-dihydroxybenzene-disulfonate (i.v.)	2186	0,90	1 - 2	1 - 2	0,25 - 1
calcium p-dihydroxybenzene-disulfonate (p.o.)	>6880	0,98	1 - 2	1 - 2	0,25 - 1
bis (diethylamine) p-dihydroxybenzene disulfonate (i.v.)	1016	2,25	1 - 2	1 - 2	0,25 - 1
bis (ammonium) p-dihydroxybenzene disulfonate	1209	2,45	1 - 2	1 - 2	0,25 - 1

For the other products of the series, the doses are within the values given above.

I claim:

Example of formulation per tablet (500 mg dose)

bis diethylamine p-dihydroxy benzene disulfonate	0.500 g	
Rice starch	0.100 g	
Lactose	0.100 g	
Polyvinylpyrrolidone	0.020 g	20
Magnesium stearate	0.003 g	
Weight of tablet	0.723 g	

Example of formulation per capsule (250 mg dose)

bis diethylamine p-dihydroxy benzene disulfonate	0.250 g	
Lactose	0.050 g	
Aerosil	0.001 g	25
Magnesium stearate	0.002 g	
Weight of capsule	0.303 g	

Example of formulation for suppositories

(500 mg dose)		
bis diethylamine p-dihydroxy benzene disulfonate	0.500 g	
Citric acid	0.0036 g	
Sodium metabisulfite	0.0002 g	30
Monolene	1.650 g	
Weight of suppository	2.15 g	

Example of formulation per injectable phial

(250 mg dose)		
bis diethylamine p-dihydroxy benzene disulfonate	0.250 g	
Sodium metabisulfite	0.002 g	35
Twice distilled water	2 ml	

1. bis (diethylamine) p-dihydroxybenzene disulfonate.

2. bis (methylamine) p-dihydroxy benzene disulfonate.

3. bis (ethanolamine p-dihydroxy benzene disulfonate.

4. bis (phenylamine) p-dihydroxy benzene disulfonate.

5. bis (piperazine) p-dihydroxy benzene disulfonate.

6. bis (dimethylamine) p-dihydroxy benzene disulfonate.

7. bis (trimethylamine) p-dihydroxy benzene disulfonate.

8. bis (pyrrolidine) p-dihydroxy benzene disulfonate.

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